cells which did exhibit these characteristic changes also exhibited strong immunohistochemical staining for CMV antigens. The atypical endothelial cells of Kaposi's sarcoma contained no immunohistochemical staining for CMV antigens (Fig 2).

The absence of CMV antigens in Kaposi's sarcoma cells implies that there is not an active infection within these cells since the antibody preparation used in these studies detected CMV antigenic material in cells known to be actively infected by this virus. The absence of active infection in Kaposi's sarcoma cells does not exclude the possibility that CMV antigenic material is present within these cells. CMV early antigens were detected by Giraldo, Beth, and Huang [4] in 7 of 31 Kaposi's sarcoma biopsy specimens; these authors also found CMV DNA sequences in 3 of 8 Kaposi's sarcoma biopsy specimens. Our results eliminate late infection of Kaposi's sarcoma cells as an explanation for the elevated antibody titers to CMV seen in patients with Kaposi's sarcoma; exposure to CMV may however be a primary event in the development of Kaposi's sarcoma.

REFERENCES

1. Giraldo G, Beth E, Kourilsky FM, Henle W, Henle G, Mike V, Huraux JM, Andersen HK, Gharbi MR, Kyalwazi SK, Puissant A: Antibody patterns to herpesviruses in Kaposi's sarcoma: Serological association of European Kaposi's sarcoma with cytomegalovirus. Int J Cancer 15:839–848, 1975
2. Giraldo G, Beth E, Henle G, Mike V, Safai B, Huraux JM, McHardy J, de-The G: Antibody patterns to herpesviruses in Kaposi's sarcoma: Serological association of American Kaposi's sarcoma with cytomegalovirus. Int J Cancer 22:126–131, 1978
3. Giraldo G, Beth E, Hahuenau F: Herpes type virus particles in tissue cultures of Kaposi's sarcoma from different regions. J Natl Cancer Institute 49:1509–1526, 1972
4. Giraldo G, Beth E, Huang E-S: The relationship of cytomegalovirus to Kaposi's sarcoma, The Human Herpesviruses. Edited by AJ Nahmias, WR Dowdle, RF Schinazi, New York, Elsevier, 1981, p 637

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Lipid Synthesis in Cutaneous Xanthoma

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***In vitro* lipogenesis was studied on the xanthoma tissue from 6 patients with normal plasma lipids and 4 patients with hyperlipidemia. Xanthoma tissue was incubated at 37°C for 6 hr in Krebs-Ringer phosphate buffer containing sodium [^{14}C]acetate. The radioactivity of each lipid class was determined after extraction and separation of lipids. The incorporation of acetate into all major lipid groups was much greater in xanthoma tissue than in control normal-appearing skin. There was no difference in the incorporation pattern of ^{14}C between xanthomas of patients with normal plasma lipids and those of hyperlipidemic patients. The data exemplify considerable in situ lipid synthesis of xanthoma tissue. Although the lipids in xanthomas of hyperlipidemic persons may be derived from plasma, the plasma origin of xanthoma lipids in normolipidemic persons remains to be confirmed, and the contribution of local lipogenesis cannot be ignored. The lipids in cutaneous xanthomas are most likely derived from a multiple input system.**

Cutaneous xanthomas may be associated with both hyperlipidemia and normal levels of blood lipids. In persons with xanthomas and hyperlipidemia, the clinical features are correlated with the plasma lipid profile [1,2], and the origin of the xanthoma lipids is believed to be circulating plasma lipoproteins, based on clinical and biochemical studies [3–5]. Evidence also has accumulated suggesting analogous mechanisms of formation of cutaneous xanthomas and atheromas [3] in persons afflicted with hyperlipidemia.

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Cutaneous xanthomas unaccompanied by hyperlipidemia occur in many clinical syndromes: histiocytosis X, diffuse plane xanthoma, xanthoma disseminatum, juvenile xanthogranuloma, and chronic lymphedema. Associations of cutaneous xanthomas with reticuloendothelial disorders and paraproteinemia have appeared frequently.

Lipid biosynthesis has been demonstrated *in vitro* in xanthomas associated with hyperlipidemia, suggesting that local formation is a significant source of lipids in the development of xanthomas [6]. To the best of our knowledge, the origin of lipids in xanthomas of persons with normal plasma lipids has not been identified. We wish to report the results of a study on lipogenesis in cutaneous xanthomas of various types from 10 patients.

CLINICAL FEATURES

Of the 10 patients with xanthomas included in this study, 6 had normal serum lipids (Table I) and 4 had hyperlipidemia (Table II).

The group with normal serum lipids had 4 types of xanthomas: xanthoma disseminatum, diffuse plane xanthoma, eruptive xanthoma, and xanthelasma palpebrarum. In 2 patients (cases 1 and 2) with xanthoma disseminatum, the lesions were generalized papular xanthomas (Fig 1*A*). Neither of those patients had diabetes insipidus or neurologic or bony abnormalities. Two patients (cases 3 and 5) had diffuse plane xanthomas (Fig 1*B*). One of these (case 5) also had hypernephroma, IgG monoclonal gammopathy, increased immature plasma cells in bone marrow, and C1-esterase inhibitor deficiency. One patient (case 6) presented with eruptive xanthomatosis, and one patient (case 4) had xanthelasma palpebrarum (Fig 1*C*).

Two patients (cases 7 and 8) had type II hyperlipoproteinemia and tuberous xanthomas (Fig 2*A*) and xanthelasmas. Three siblings of patient 7 had died of cardiovascular disease. Patient 8 had a sibling who died of heart disease at the age of 7 yr; and both of his parents and another sibling had elevated levels of blood lipids. Patient 9 presented anomalous hyperlipoproteinemia characterized mainly by increased β very-low-density lipoproteins of atypical composition, and patient 10 had type V hyperlipoproteinemia. Patient 9 presented with diffuse

TABLE I. *Clinical data on 6 patients with xanthomas and normal levels of serum lipids*

Case	Sex and age (yr)	Plasma lipid (mg/dl)			Xanthoma lesions	Duration of lesions (yr)	Diagnosis
		Cholesterol	Triglycerides	Phospholipids			
1	F, 47	218	62	246	Generalized papular	½	Xanthoma disseminatum
2	F, 47	161	114	168	Generalized papular	10	Xanthoma disseminatum
3	F, 72	191	66	224	Generalized plane	15	Diffuse plane xanthoma
4	F, 43	229	122	230	Xanthelasma	5	Xanthelasma palpebrarum
5	M, 71	156	40	160	Generalized plane	(?)	Diffuse plane xanthoma
6	M, 29	150	127	···	Eruptive	(?)	Eruptive xanthomatosis

TABLE II. *Clinical data on 4 patients with xanthoma and hyperlipidemia*[a]

Case	Sex and age (yr)	Plasma lipid (mg/dl)			Plasma lipoprotein profile				Type	Xanthoma lesion
		Cholesterol	Triglycerides	Phospholipids	β	Pre-β	α	Chylomicron		
7	M, 47	514	325	155	Large increase	Small increase	Normal	Normal	IIB	Tuberous xanthoma and xanthelasma
8	M, 17	846	161	500	Very large increase	Normal	Normal	Normal	IIA	Tuberous xanthoma and xanthelasma
9	F, 61	297	242	···	Normal LDL, increased VLDL	Normal	Normal	Small increase	Anomalous increase of β-VLDL	Diffuse plane xanthoma, multiple myeloma
10	M, 36	500	4,780	···	Normal	Very large increase	Normal	Large increase	V	Eruptive xanthoma

[a] VLDL and LDL refer to very-low-density lipoproteins and low density lipoproteins.

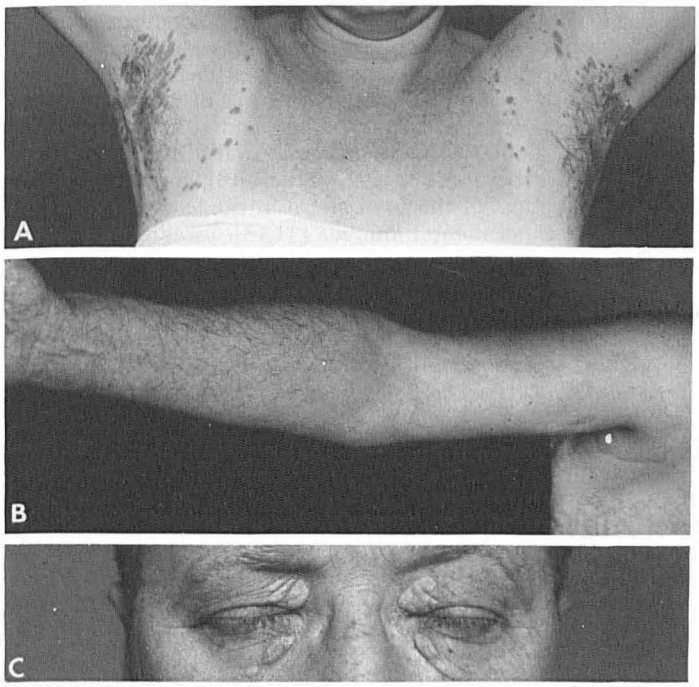

FIG 1. Xanthomas from persons with normal levels of lipids in serum. *A*, Papular xanthoma with generalized distribution. *B*, Diffuse plane xanthoma. *C*, Xanthelasma palpebrarum.

plane xanthomatosis, and patient 10 presented with eruptive xanthomatosis (Fig 2*B*). In addition, patient 9 was found to have multiple myeloma. There was no history of cardiovascular disease in the families of these 2 patients.

Xanthomatous lesions in all of the 10 patients were documented by histologic evidence of dermal foamy fat-laden xanthoma cells.

MATERIALS AND METHODS

From each patient, after an overnight fast, specimens of xanthoma tissue and normal-appearing skin from the buttocks were obtained by a 6-mm punch biopsy or an excisional biopsy under local anesthesia (lidocaine). Each specimen was dissected free of adipose tissue and immediately blotted and weighed. The weights of the specimens ranged from 29.4 to 51.7 mg. Each specimen was then incubated in 2 ml of Krebs-Ringer phosphate buffer (pH 7.4) containing 3.3 μCi of sodium [^{14}C]acetate (1.66 μmol), 200 units of penicillin, 0.2 mg of streptomycin, and 80 mg of glucose for 6 hr at 37°C in a Dubnoff metabolic shaking incubator. At the end of incubation, the specimen was removed from the incubation medium and rinsed with distilled water. Each skin specimen was blotted between filter papers and homogenized in 10 ml of chloroform:methanol (2:1, vol:vol). The solid debris was removed by filtration and washed three times with 5 ml of chloroform:methanol (2:1, vol:vol). The incubation medium was taken to dryness under reduced pressure with a rotary evaporator; 0.1 ml of glacial acetic acid was added, and the mixture was dried in the evaporator by gentle heating (40°C). The residue was extracted with 5 ml of chloroform:methanol (2:1, vol:vol). The lipids that were extracted from tissue specimens and from the incubation medium were fractionated separately by thin-layer chromatography in silica gel H. The chromatograms were developed with chloroform:methanol:water (14:6:1, vol:vol:vol). The lipid bands were located by means of iodine vapor and identified by chromatographic mobilities (R_f). Fractions of phospholipids, fatty acids, and triglycerides were eluted with 10 ml of methanol containing 1% dry HCl. (The HCl in the solvent made possible the quantitative elution of the phospholipids.) Fractions of cholesterol and cholesteryl esters were eluted with 10 ml of chloroform:methanol (2:1, vol:vol). After evaporation of the solvents from counting vials, the lipids were redissolved in 15 ml of toluene, and 1 ml of scintillation solution was added to each vial. The radioactivity was measured with a liquid scintillation spectrometer (Packard Tri-Carb model 3002). The incorporation of lipids was expressed as counts per minute per gram of tissue.

In 4 cases (cases 5, 6, 9, and 10), the xanthoma tissue and normal-appearing skin were rinsed with distilled water after being removed from the incubation medium at the end of incubation. The specimens were soaked in 2 M NaI solution at 37°C for 30 min, and the dermis was separated from the epidermis. Each epidermis-free specimen was homogenized, and the lipids were extracted by the procedure already described for whole skin.

RESULTS

In the xanthoma specimens, acetate was incorporated into the lipids of all the major classes (Tables III and IV). The

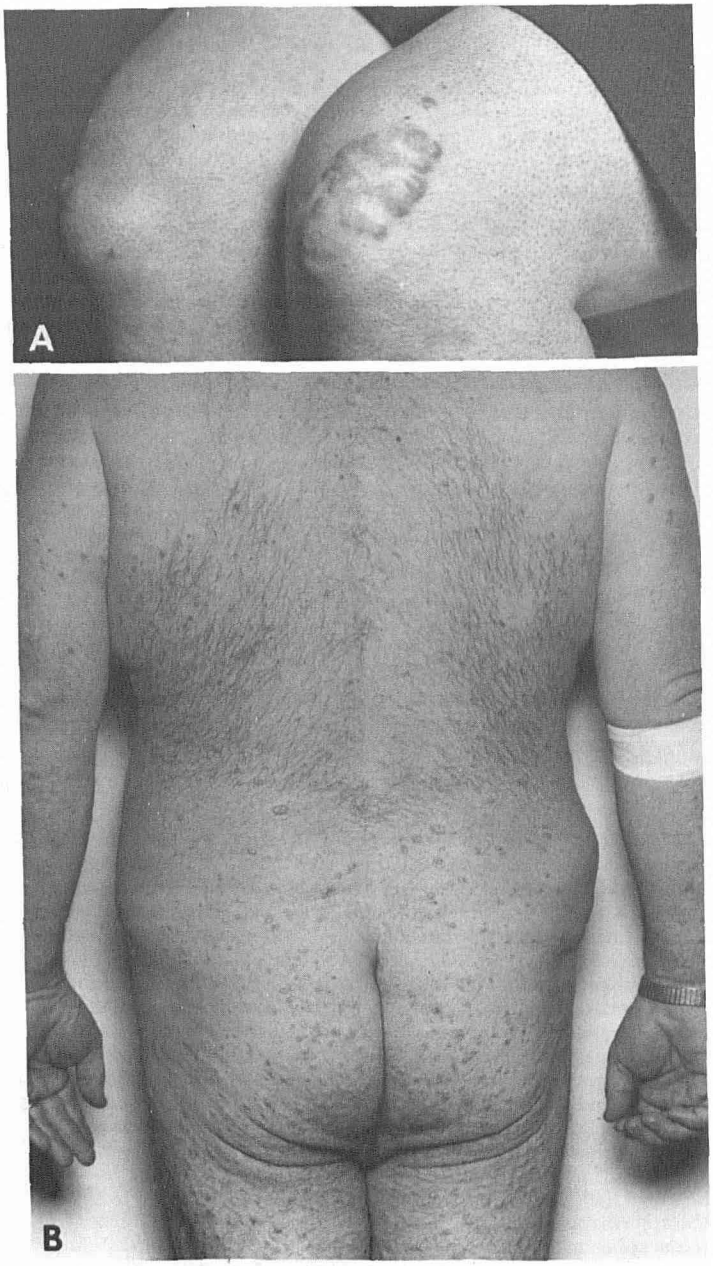

FIG 2. Xanthomas from persons with hyperlipidemia. *A*, Tuberous xanthoma. *B*, Eruptive xanthoma.

release of lipids into the incubation medium was negligible. On the basis of tissue weight, the xanthomas showed a 1.2- to 48.7-fold incorporation of acetate into all lipid groups in comparison with normal-appearing skin. There was no significant difference of increased incorporation between xanthomas from patients with normal serum lipids and xanthomas from hyperlipidemic patients. The greatest incorporation occurred in cholesteryl ester and phospholipid fractions.

The incorporation of labeled acetate into lipids of all classes was greater in xanthoma tissue (cases 5, 6, 9, and 10) than in the normal-appearing skin from the same donor when the results were expressed in terms of cpm incorporated per gram of tissue. The actual numbers of micromoles of the various lipids synthesized per unit of tissue during the incubations remain unknown, because the specific activities of the acetate within the specimens could not be determined with certainty.

The relative increase of incorporation was greatest in phospholipids; the incorporation of acetate into xanthoma phospholipids was 1.8- to 48.7-fold greater than the incorporation into phospholipids by normal skin.

DISCUSSION

The results of the present study show that local lipid synthesis occurs in cutaneous xanthomas from persons with hyperlipidemia and from persons with normal serum lipids.

Based on the following observations, it has been hypothesized that the lipids in cutaneous xanthomas associated with hyperlipidemia are derived from plasma lipoproteins. First, cholesteryl ester-fatty acid patterns have been found to be similar in the plasmas and in the xanthomas of cholesterol-fed rabbits [3]. Second, electron microscopic study has shown that lipoproteins may permeate the vessel wall [4]. Third, radioisotopic tracer studies have demonstrated the existence of plasma lipoproteins in the cutaneous lesions [5]. However, an additional source of lipids was indicated in one study [6], in that active local biosynthesis of phospholipids and free fatty acids and of extremely small amounts of cholesterol were demonstrated in xanthoma tissue from a patient with hyperlipidemia. Our data agree with those findings, and in addition, we have demonstrated significant synthesis of cholesterol and cholesteryl esters in xanthoma tissue. This additional observation may have been permitted by a longer duration of *in vitro* incubation and a greater specific activity of substrate in our experiments. That these 2 factors could contribute to the difference in incorporation was demonstrated and discussed by Hsia, Sofer, and Lane [7]. Significant incorporation into all lipids occurs by 3 hr; the incorporation into fatty acids reaches a plateau after 9 hr. The incorporation into squalene and sterols continues after 20 hr.

Ample studies support the attractive hypothesis that atheromas and xanthomas of persons with hyperlipidemia are formed by analogous mechanisms. Morphologic similarities were observed at light microscopic levels several decades ago [8]. A progression of pericytes into foam cells has been demonstrated ultrastructurally in both xanthomas and atheromas [9–13]. Biochemical similarities of xanthomas and atheromas include cholesterol as the major tissue lipid [14–16], cholesteryl esters contributing most of the total sterol in the lesions in humans [16,17] and experimental animals [11,18]. In addition, similar cholesteryl ester-fatty acid profiles have been found in the plasma, xanthomas, and atheromas both in humans [19] and in experimental animals [4,20–23]. Although the lipids in atheromas, as in xanthomas associated with hyperlipidemia, are believed to be predominantly of plasma origin [3,20–26], active lipogenesis has been demonstrated in atheromas of the rabbit and pigeon [27–30] and in human fatty streak lesions of aorta, with the greatest relative increase of lipid occurring in the cholesteryl ester fraction [31]. Day, Wahlqvist, and Tume [32–34] have demonstrated that foam cells may be an important site of lipid synthesis in rabbit and human atherosclerotic lesions. Smith and Slater [35] suggested that lipids in normal intima are derived primarily from plasma lipoproteins, whereas much of the lipids of fatty plaques may have a local origin. Large increases in rates of cholesterol esterification were found in the atheromatous lesions of cholesterol-fed rabbits, as compared with normal vessel sections. The local synthesis has been estimated to account for 50% of the cholesteryl esters that accumulate [36].

Local lipogenesis contributing to tissue phospholipids has been evident in xanthomas [5] from persons with hyperlipidemia and in atheromas [37–39].

In a study on long-term kinetics of serum and xanthoma cholesterol radioactivity in hypercholesterolemic patients, Samuel et al [40] pointed out the possibility of local lipid synthesis. In one of their 4 cases, after the intravenous injection of [^{14}C]cholesterol, the xanthoma curve representing [^{14}C]cholesterol intersected the serum curve after the xanthoma curve had reached its maximum. This result indicated that a multiple input system might have been operating and that the lipids in

TABLE III. *Incorporation of [^{14}C]acetate into lipids by xanthomas from 6 patients with normal levels of serum lipids*

	Case 1	Case 2	Case 3	Case 4	Case 5[a]	Case 6[a]
Cholesteryl ester (cpm/g)						
Normal skin	7,445	4,441	12,842	10,409	4,476	9,935
Xanthoma tissue	55,223	29,516	35,017	31,889	64,371	56,639
Cholesteryl (cpm/g)						
Normal skin	2,293	2,738	5,660	8,996	2,447	3,472
Xanthoma tissue	16,810	13,022	22,424	22,309	14,679	32,136
Free fatty acids (cpm/g)						
Normal skin	4,239	3,844	5,099	5,909	2,669	527
Xanthoma tissue	11,720	12,421	8,734	14,687	7,445	17,142
Triglycerides (cpm/g)						
Normal skin	1,278	2,509	7,649	5,393	1,033	2,950
Xanthoma tissue	6,500	19,831	23,947	15,669	6,775	16,479
Phospholipids (cpm/g)						
Normal skin	1,255	4,466	3,017	2,153	2,093	542
Xanthoma tissue	42,687	51,784	5,808	13,233	16,244	26,437
Total lipids (cpm/g)						
Normal skin	16,500	18,000	34,000	33,000	13,000	17,400
Xanthoma tissue	133,000	127,000	96,000	98,000	110,000	149,000
Ratio (xanthoma/normal)	8	7	3	3	8	9
Total lipids (μmol Ac incorporated)						
Normal skin	0.0037	0.0041	0.0077	0.0075	0.0029	0.0039
Xanthoma tissue	0.0301	0.0287	0.0217	0.0222	0.0249	0.0337
Lipids formed (μg/24 h)						
Normal skin	0.89	0.98	1.85	1.80	0.70	0.94
Xanthoma tissue	7.22	6.89	5.21	5.33	5.98	8.09
Ratio (xanthoma/normal)	8	7	3	3	8	9

[a] Epidermis removed by NaI.

TABLE IV. *Incorporation of [^{14}C]acetate into lipids by xanthomas from 4 patients with hyperlipidemia*

	Case 7	Case 8	Case 9[a]	Case 10[a]
Cholesteryl ester (cpm/g)				
Normal skin	7,429	13,674	3,238	3,237
Xanthoma tissue	21,437	59,647	41,990	96,432
Xanthelasma	17,321	41,993	...	...
Cholesteryl (cpm/g)				
Normal skin	6,479	4,239	4,592	7,227
Xanthoma tissue	21,730	17,204	19,247	84,362
Xanthelasma	13,021	9,610	...	...
Free fatty acids (cpm/g)				
Normal skin	3,295	5,716	665	2,669
Xanthoma tissue	4,189	11,788	12,746	7,445
Xanthelasma	6,243	9,973	...	...
Triglycerides (cpm/g)				
Normal skin	1,047	6,231	449	602
Xanthoma tissue	2,977	18,727	10,529	11,375
Xanthelasma	4,769	10,473	...	...
Phospholipids (cpm/g)				
Normal skin	3,024	1,927	689	7,325
Xanthoma tissue	31,274	21,001	25,976	29,742
Xanthelasma	17,493	12,365	...	...
Total lipids (cpm/g)				
Normal skin	21,000	32,000	9,600	21,000
Xanthoma tissue	82,000	128,000	110,000	229,000
Ratio (xanthoma/normal)	4	4	11	11
Total lipids (μmol Ac incorporated)				
Normal skin	0.0048	0.0072	0.0022	0.0047
Xanthoma tissue	0.0186	0.0290	0.0249	0.0518
Lipids formed (μg/24 h)				
Normal skin	1.15	1.73	0.53	1.13
Xanthoma tissue	4.46	6.96	5.98	12.43
Ratio (xanthoma/normal)	4	4	11	11

[a] Epidermis removed by NaI.

hyperlipidemic cutaneous xanthomas might have been derived both from plasma and from local synthesis.

Our data provide evidence that local synthesis of the major lipids, including cholesterol and cholesteryl esters, occurs in xanthomas associated with hyperlipidemia. We also demonstrated the potentiality of local lipogenesis in cutaneous xanthomas of persons who have normal plasma lipids.

The incorporation of ^{14}C into the sum of the cholesterol, cholesteryl esters, fatty acids, triglycerides, and phospholipids (Tables III and IV) ranged from a minimum of 9,600 cpm/g tissue per 6 hr of incubation for "normal skin" to a maximum of 229,000 cpm/g tissue per 6 hr for xanthomas. Those values translate into approximately 0.0022 to 0.0518 μmol of exogenous substrate incorporated and approximately 0.53 to 6.0 μg of lipid

formed per 24 hr (projected). Those quantities of [^{14}C]acetate incorporated and lipid formed are surprisingly large, considering that the [^{14}C]acetate was presented to the tissue specimens quite disadvantageously in terms of the geographic relationship to the biosynthesis apparatus of the specimens and that lipid formation from endogenous substrate probably exceeded lipid formation from the exogenous acetate by several-fold.

Two observations indicate that the incorporation of ^{14}C into the lipids represents lipogenesis and not simply "lipid modification processes" or "isotope exchange processes." First, the incorporation into cholesterol could represent only de novo synthesis, because in mammalian tissues acetate is incorporated only in early stages of the cholesterogenic process, and carbon from cholesterol cannot be exchanged with carbon from acetate or from any other substance. Second, in two unreported preliminary incubations, one of the authors (RDE) observed that 80 to 86% of the ^{14}C (from acetate) in the fatty acids was contained in the palmitate fraction. (Fractions were separated with an F & M Model 500 gas chromatograph equipped with a katharometer detector and were collected in acetone.) Because the biosynthesis of palmitate is achieved mainly by the palmitate synthase system and not by an add-on process involving ^{14}C substrate and because, like cholesterol, palmitate does not participate in carbon-exchange reactions with other substances, the incorporation of [^{14}C]acetate into palmitate must have represented de novo lipogenesis. While we did not examine the distribution of ^{14}C among the individual fatty acids from the specimens evaluated in this report, we believe that the ^{14}C of the fatty acids was contained mainly in palmitate, as it was in our preliminary study, and that it represented true lipogenesis.

On the basis of per gram of wet tissue, the xanthoma specimens incorporated from 3 to 11 times as much ^{14}C into the lipids as did the respective control specimens. On the basis of the residual weights after extraction of the lipids from the specimens, the differences would be much greater; however, we did not weigh the residual proteins.

Our data strongly indicate that lipogenesis is much more active in xanthomas than in "normal" skin. Further exploration should be directed toward identification of the type(s) of cell(s) responsible for this difference—perhaps aberrant dermal epithelial cells, aberrant perithelial cells, or macrophages. Nevertheless, our data suggest that lipogenesis in situ, regardless of the cell type(s) responsible, is a significant source of xanthoma lipids.

Whether plasma lipids contribute to the formation of cutaneous xanthomas in the absence of hyperlipidemia is not clear; presumably, plasma lipid deposition in xanthomas can occur when plasma lipid levels are normal. From previous studies and the data in our present study, it is reasonable to conclude that, in persons with either normal or elevated plasma lipid levels, xanthoma lipids are derived from a multiple input system in which local lipogenesis can be a very significant factor. The significance of the observed biosynthesis of lipids in xanthoma tissue remains unknown. This lipogenic activity might very well be primarily an expression of the biosynthesis of membranous structures in a proliferating tissue.

REFERENCES

1. Parker F, Short JM: Xanthomatosis associated with hyperlipoproteinemia. J Invest Dermatol 55:71–88, 1970
2. Polano MK, Baes H, Hulsmans HAM, Querido A, Pries C, van Gent CM: Xanthomata in primary hyperlipoproteinemia: a classification based on the lipoprotein pattern of the blood. Arch Dermatol 100:387–399, 1969.
3. Parker F, Peterson N, Odland GF: A comparison of cholesterol-ester fatty acid patterns in the blood and in evolving xanthoma and atheroma during cholesterol-feeding of rabbits. J Invest Dermatol 47:253–259, 1966
4. Parker F, Bagdade JD, Odland GF, Bierman EL: Evidence for the chylomicron origin of lipids accumulating in diabetic eruptive xanthomas: A correlative lipid biochemical, histochemical, and electron microscopic study. J Clin Invest 49:2172–2187, 1970.
5. Scott PJ, Winterbourn CC: Low-density lipoprotein accumulation in actively growing xanthomas. J Atheroscler Res 7:207–223, 1967
6. Wilson JD: Studies on the origin of the lipid components of xanthomata. Circ Res 12:472–478, 1963
7. Hsia SL, Sofer G, Lane B: Lipid metabolism in human skin. I. Lipogenesis from acetate-1-^{14}C. J Invest Dermatol 47: 437–442, 1966
8. Plewes LW: Nature and origin of the xanthoma cell. Arch Pathol 17:177–186, 1934.
9. Geer JC, McGill HC Jr, Strong JP: The fine structure of human atherosclerotic lesions. Am J Pathol 38:263–287, 1961
10. Balis JU, Haust MD, More RH: Electron-microscopic studies in human atherosclerosis cellular elements in aortic fatty streaks. Exp Mol Pathol 3:511–525, 1964
11. Parker F, Odland GF: A correlative histochemical, biochemical and electron microscopic study of experimental atherosclerosis in the rabbit aorta with special reference to the myo-intimal cell. Am J Pathol 48:197–239, 1966
12. Ghidoni JJ, O'Neal RM: Recent advances in molecular pathology: A review; ultrastructure of human atheroma. Exp Mol Pathol 7:378–400, 1967.
13. Parker F, Odland GF: Experimental xanthoma: a correlative biochemical, histologic, histochemical, and electron microscopic study. Am J Pathol 53:537–565, 1968
14. Montgomery H, Osterberg AE: Xanthomatosis: correlation of clinical, histopathologic and chemical studies of cutaneous xanthoma. Arch Dermatol Syphilol 37:373–401, 1938
15. Rabinowitz JL, Riemenschneider RW: Lipid composition and lipogenesis of intimal areas from a single human aorta. Lab Invest 12:549–553, 1963
16. Fletcher RF, Gloster J: The lipids in xanthomata. J Clin Invest 43:2104–2111, 1964
17. Böttcher CJF, Woodford FP: Chemical changes in the arterial wall associated with atherosclerosis. Fed Proc 21(suppl 2):15–19, 1962
18. Wang C-I, Strauss L, Adlersberg D: Experimental xanthomatosis in rabbits. Arch Pathol 63:416–422, 1957
19. Smith EB, Evans PH, Downham MD: Lipid in the aortic intima: the correlation of morphological and chemical characteristics. J Atheroscler Res 7:171–186, 1967
20. Evrard E, Van den Bosch J, Joossens JV, De Somer P: Fatty acid composition of plasma lipids of normal, triton-treated and cholesterol-fed rabbits. Am J Clin Nutr 10:240–249, 1962
21. Billiau A, Evrard E, Van den Bosch J, Joossens JV, De Somer P: The fatty acid distribution in plasma, liver and aorta of cholesterol-fed and triton-treated rabbits. J Atheroscler Res 3:222–232, 1963
22. Chalvardjian AM, Still WJS: Fatty acid composition of tissues in cholesterol-fed rabbits. J Atheroscler Res 4:507–516, 1964
23. Biggs MW, Kritchevsky D: Observations with radioactive hydrogen (H^3) in experimental atherosclerosis. Circulation 4:34–42, 1951
24. Evrard E, Van den Bosch J, De Somer P, Joossens JV: Cholesteryl ester fatty acid patterns of plasma, atheromata and livers of cholesterol-fed rabbits. J Nutr 76:219–222, 1962
25. Schwenk E, Stevens DF: Deposition of cholesterol in experimental rabbit atherosclerosis. Proc Soc Exp Biol Med 103:614–617, 1960
26. Newman HAI, Zilversmit DB: Quantitative aspects of cholesterol flux in rabbit atheromatous lesions. J Biol Chem 237:2078–2084, 1962
27. Feller DD, Huff RL: Lipide synthesis by arterial and liver tissue obtained from cholesterol-fed and cholesterol-alcohol-fed rabbits. Am J Physiol 182:237–242, 1955
28. McCandless EL, Zilversmit DB: The effect of cholesterol on the turnover of lecithin, cephalin and sphingomyelin in the rabbit. Arch Biochem Biophys 62:402–410, 1956
29. Whereat AF: Lipid biosynthesis in aortic intima from normal and cholesterol-fed rabbits. J Atheroscler Res 4:272–282, 1964
30. Lofland HB Jr, Moury DM, Hoffman CW, Clarkson TB: Lipid metabolism in pigeon aorta during atherogenesis. J Lipid Res 6:112–118, 1965
31. Chobanian AV, Manzur F: Metabolism of lipid in the human fatty streak lesion. J Lipid Res 13:201–206, 1972
32. Day AJ, Wahlqvist ML: Uptake and metabolism of ^{14}C-labeled oleic acid by atherosclerotic lesions in rabbit aorta: a biochemical and radioautographic study. Circ Res 23:779–788, 1968
33. Wahlqvist ML, Day AJ, Tume RK: Incorporation of oleic acid into lipid by foam cells in human atherosclerotic lesions. Circ Res 24:123–130, 1969
34. Day AJ, Tume RK: *In vitro* incorporation of ^{14}C-labelled oleic acid into combined lipid by foam cells isolated from rabbit atheromatous lesions. J Atheroscler Res 9:141–149, 1969
35. Smith EB, Slater RS: The lipoproteins of the lesions, International Symposium on Atherosclerosis, 2nd edition, Chicago, 1969. Edited by RJ Jones. New York, Springer-Verlag, 1970, pp 42–49.
36. Dayton S, Hashimoto S: Origin of fatty acids in lipids of experimental rabbit atheroma. J Atheroscler Res 8:555–568, 1968
37. Zilversmit DB, Shore ML, Ackerman RF: The origin of aortic phospholipid in rabbit atheromatosis. Circulation 9:581–585, 1954
38. Zilversmit DB, McCandless EL: Independence of arterial phospholipid synthesis from alterations in blood lipids. J Lipid Res 1:118–124, 1959–1960
39. Newman HAI, McCandless EL, Zilversmit DB: The synthesis of

C^{14}-lipids in rabbit atheromatous lesions. J Biol Chem 236:1264–1268, 1961
40. Samuel P, Perl W, Holtzman CM, Rochman ND, Lieberman S: Long-term kinetics of serum and xanthoma cholesterol radioactivity in patients with hypercholesterolemia. J Clin Invest 51:266–278, 1972

0022-202X/82/7902-0085$02.00/0
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Effect of Chronologic Aging and Ultraviolet Irradiation on Langerhans Cells in Human Epidermis

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The effect of aging on epidermal Langerhans cells (LC) and on their response to a single ultraviolet (UV) exposure was studied in skin biopsy specimens of healthy adults, 4 aged 22–26 yr and 7 aged 62–86 yr. In unirradiated skin, old adults had fewer LC than young adults, 5.8 ± 1.1 versus 10.0 ± 0.8 (mean ± SEM) per 3 mm wide cross-section ($p = .015$). Following irradiation with 3 times the minimal erythema dose, recognizable LC were absent in all but 2 subjects within 24 hr. However, LC number fell less rapidly in old adults and was almost unchanged at 4 hours (5.8 ± 1.1 versus 5.0 ± 1.2), while in young adults LC number decreased from 10.0 ± 0.8 to 3.3 ± 1.3 during the same period ($p < .05$). Other changes noted in both young and old subjects following irradiation included cytoplasmic vacuolization, frequent apposition of LC to severely damaged keratinocytes, and the finding of LC in the basal layer of the epidermis rather than exclusively suprabasilarly as in control sections. These data demonstrate an age-associated loss of epidermal LC and slowing of LC response to UV irradiation. UV-induced LC changes appear qualitatively similar in young and old adults and include histologic evidence of cellular damage, transient association of LC with damaged keratinocytes, and possible migration of LC from the irradiated epidermis within 24 hr.

Over the past decade much progress has been made toward understanding the function of Langerhans cells (LC), a dendritic subpopulation in the epidermis [1]. Recent studies have shown LC to be of bone marrow origin [2], to have surface receptors for immunoglobulin (Fc-IgG) and complement (C3b) [3], to express B cell alloantigens (Ia) [4,5], to preferentially accumulate cutaneously applied antigens [6], to stimulate antigen specific T cell activation [7], and to migrate between hematopoietic tissues and the skin [2]. These observations have led to the hypothesis that these cells represent the "farthest outpost" of the body's immune surveillance system and that they are necessary participants in the processing of antigens presented to the skin.

Two early electron microscopic studies [8,9] suggested UV irradiation could decrease the number of identifiable LC in skin. Recently Toews, Bergstresser, and Streilein reported that 4 daily UV exposures greatly reduced the number of LC as recognized by ATPase surface markers in the abdominal skin of mice and that the irradiated, LC-depleted skin was incapable of initiating a delayed hypersensitivity response to DNCB [10]. Aberer et al subsequently reported that in both human and mouse skin a single exposure to midrange (290–320 nm) UV reduced LC as recognized either by Ia and ATPase surface markers or by electron microscopic criteria [11].

We now report data which confirm and quantify this effect of UV irradiation on LC in human skin and which provide the first evidence that chronologic aging is also associated with a reduction of this epidermal cell population.

MATERIALS AND METHODS

Subjects

Eleven volunteers, 4 aged 22–26 yr (mean 23 yr, 2 male/2 female) and 7 aged 62–86 yr (mean 69 yr, 2 male/5 female), enrolled in the study after giving informed consent. All subject were fair-skinned Caucasians in excellent health, used no mediations except multivitamins, and had no history of skin disease or abnormal reactions to sun exposure.

UV Radiation

A Hanovia hot quartz mercury vapor lamp was used. Approximately 80% of the lamp output lies above 285 nm [12] and most of a moderate human erythema response may be attributed to this portion of the spectral output.

The minimal erythema dose (MED) was determined on the previously nonsunexposed buttock skin of each subject using 6 incremental exposures of 15 mj/cm^2 (15–90 mj/cm^2) through 1 × 2 cm portals. Erythema was graded visually at 24 hr: 0 = absent, ± = faint without distinct borders, 1+ = faint with 4 distinct borders (MED), 2+ = moderate to intense macular, 3+ = intense with edema, 4+ = with blister formation.

Histology Techniques

Skin biopsies 3 mm in diameter were obtained after the site was anesthetized with 2% lidocaine without epinephrine. Each specimen was placed immediately in chilled Karnovsky's mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer, postfixed in osium tetroxide, embedded in Epon, cut to one micrometer in thickness with a Sorvall MT—1 microtome, stained with Giemsa, and examined with a light microscope. Each specimen was coded at the

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Abbreviations:
LC: Langerhans cells